



An integrative approach to species delimitation sinks three Chinese limestone karst *Elatostema* (Urticaceae) species

Zi-Bing Xin¹, Alexandre K. Monroe², Ren-Fen Wang¹, Long-Fei Fu¹

¹ Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin 541006, China

² Identification & Naming Department, Royal Botanic Gardens, Kew TW9 3AE, UK

Corresponding author: Long-Fei Fu (longfeifu@126.com)

Abstract

Elatostema is recognized as a taxonomically difficult group due to the reduced nature of the tiny flowers and inflorescences, also the large number of species (ca 650 to 700). Different opinions on morphological species delimitation have resulted in instability, which is problematic in such a speciose group. In this paper, the taxonomic status of three putative species, *E. robustipes*, *E. scaposum*, *E. conduplicatum* and their hypothetical closest relatives, was revised using morphological and molecular observations. Morphological comparison suggested high similarity between *E. robustipes* & *E. retrohirtum*, *E. scaposum* & *E. oblongifolium*, *E. conduplicatum* & *E. coriaceifolium*, respectively. Phylogenetic analyses of four universal DNA barcodes (ITS, *trnH-psbA*, *matK* and *rbcL*) suggested that each species pair represents a single evolutionary lineage. Taking these two findings together, we propose *E. robustipes* to be a synonym of *E. retrohirtum*, *E. scaposum* a synonym of *E. oblongifolium*, and *E. conduplicatum* a synonym of *E. coriaceifolium*. Our results recover the number, shape and size of the bracts and bracteoles to be relatively stable characters, and the disposition of the male inflorescences on modified stems to be an unstable character, unsuitable for species delimitation in *Elatostema*.

Key words: DNA barcoding, integrative taxonomy, phylogenetically informative morphological characters, phylogeny, point-endemics



Academic editor: Clifford Morden

Received: 28 October 2023

Accepted: 21 November 2023

Published: 6 December 2023

Citation: Xin Z-B, Monroe AK, Wang R-F, Fu L-F (2023) An integrative approach to species delimitation sinks three Chinese limestone karst *Elatostema* (Urticaceae) species. *PhytoKeys* 236: 83–96. <https://doi.org/10.3897/phytokeys.236.114837>

Copyright: © Zi-Bing Xin et al.

This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

Introduction

Elatostema J.R.Forst. & G.Forst. is one of the most species-rich genera in the Urticaceae, comprising ca 650 to 700 species of mainly succulent herbs that grow in dense shade of forest, stream sides, gorges, and caves (Wang 2014; POWO 2023). *Elatostema* is distributed throughout tropical and subtropical Africa, Madagascar, Asia, Australia and Oceania (Lin et al. 2003). Recent phylogenetic studies suggest that *Elatostema* is a monophyletic group that includes taxa hitherto attributed to *Pellionia*, but excludes those attributed to *Elatostematoides*, *Procris*, and *Pellionia repens* (Tseng et al. 2019).

Southwest China and Southeast Asia are renowned biodiversity hotspots, in part due to extensive limestone karst present in both (Xu 1995; Clements

et al. 2006; Wei 2018; Wei et al. 2022). Southwest China is the center of both *Elatostema* species diversity and morphological variation within the genus, which suggests that it may be a center of diversification for the genus (Wang 2014). This diversity is associated with limestone karst, a fragile habitat characterized by substantial exposed rock, shallow soils deficient in N and P, excessive Ca, Mg, seasonal droughts (Hao et al. 2015), high species diversity and point-endemism (Clements et al. 2006). Karst has been subject to extensive human intervention (mining, agriculture) and is very sensitive to climate change and water pollution (Parise et al. 2009; Jiang et al. 2014). Documenting this biodiversity is a high priority if species are to be conserved or their extinction threat assessed (Clements et al. 2006; Wang 2014). Collecting in karst is, however, very difficult as there are few roads and the terrain is irregular and steeply dissected. A consequence of the above is that there are relatively few collections from such areas and undescribed species are frequently represented by one or two collections only (Fu et al. 2019). Describing species based on few collections is problematic and carries the risk of over-describing species (Fu et al. 2019). Furthermore, considering the information lacking on pistillate inflorescence (21%) and staminate inflorescence (29%) of Chinese *Elatostema* species (Wang 2014), as well as the extensive occurrence of apomixis in *Elatostema* (Fu et al. 2017), it is likely that many new species are described from populations that comprise a single sex, further increasing the risk of over-description, whereby male and female populations can be described as different species.

Over the past two decades, there has been some instability in *Elatostema* species delimitation in China with several *Elatostema* species being placed in synonymy or reduced to ranks below that of species (Duan and Lin 2003; Lin and Duan 2003; Duan et al. 2006; Lin et al. 2011) and then raised back to species rank (Wang 2010a, 2014) because of different opinions on the importance of specific morphological characters. Wang (2010a) studied bract and bracteole morphology of *Elatostema* and concluded that their number, shape and size are relatively constant. More recently, additional morphological characters, such as male inflorescences borne on modified stems, were identified and used to delimitate species (Yang et al. 2011; Wang 2014). The phylogenetic informativeness of these characters has not, however, been evaluated (Lin et al. 2011). Controversy in species delimitation cannot, therefore, be resolved by morphological evidence alone.

Phylogenetic analyses of DNA barcodes can provide a means to use paraphyly to identify conspecific groupings and it has been successfully applied to delimit *Elatostema setulosum* W.T.Wang (Fu et al. 2019).

In this study we evaluate the taxonomic status of three putative *Elatostema* species, *E. robustipes* W.T.Wang, F.Wen & Y.G.Wei, *E. scaposum* Q.Lin & L.D.Duan, *E. conduplicatum* W.T.Wang. To do so, we employed four universal DNA barcodes (ITS, *trnH-psbA*, *matK* and *rbcL*) (Hollingsworth et al. 2009, 2011; China Plant BOL Group et al. 2011) and visual morphological comparison. We aimed to use a phylogenetic framework to evaluate the phylogenetic informativeness of several morphological characters (see Wells et al. 2021) that have previously been used for species delimitation in the above species, as well as assess their monophyly.

Materials and methods

Taxon sampling

Fieldtrips in SW China and northern Vietnam were conducted between 2009 and 2023 to collect specimens and DNA materials of *Elatostema robustipes*, *E. scaposum*, *E. conduplicatum* and their closest relatives for morphological and molecular studies. Our sampling strategy of molecular analysis aimed to sample all putative species and their closest relatives, using material collected from type localities or, where this was not possible, to sample material that exhibited the diagnostic morphological characters for those species. *Elatostema robustipes* and *E. conduplicatum* were collected from type localities and *E. scaposum* from a specimen with key characters. The studied material covered the main distribution range of the respective species in SW China to northern Vietnam. In total, seven species with 11 accessions represented by three putative species and their closest relatives with the exception of *E. shanglinense* W.T.Wang (DNA material is not available) were ingroups. *Elatostema radicans* (Siebold & Zucc.) Wedd. and *E. heterolobum* (Wedd.) Hallier f. were selected as outgroups based on previous phylogenetic analyses (Tseng et al. 2019). Materials for molecular analysis are listed in Table 1. The morphological comparison between putative species and their closest relatives were based on consulting protologues, checking type specimens and other specimens as well as observing field individuals. Specimens used for morphological studies are listed in Appendix 1.

Morphological examination

A morphological species concept was employed to compare the taxa based on Wei et al. (2011). Specimens were examined using dissecting microscopy following Fu et al. (2019). The selection of morphological characters was

Table 1. Species name, voucher specimen of *Elatostema* and their accession numbers of ITS, *trnH-psbA*, *matK* and *rbcl* used in this study (*denoted newly generated sequences).

Species name	Accession	Voucher specimen	Locality	ITS	<i>trnH-psbA</i>	<i>matK</i>	<i>rbcl</i>
<i>Elatostema balansae</i>	C098	L.F. Fu & S.L. Huang FL0001 (IBK)	Yunnan, Guangxi	OR733575*	OR730813*	OR730814*	OR730815*
<i>E. conduplicatum</i>	C038	L.F. Fu FLF042 (IBK)	Guangxi, China	OR577332*	OR568594*	OR568577*	OR591476*
<i>E. coriaceifolium</i>	C696	F. Wen 0097 (IBK)	Guizhou, China	OR577335*	OR568595*	OR568585*	OR591473*
	C140	F. Wen WF0070 (IBK)	Guizhou, China	OR577334*	OR568596*	OR568583*	OR591468*
	J078	Z.B. Xin XZB20180128-01 (IBK)	Guangxi, China	OR577333*	OR568597*	OR568584*	OR591475*
<i>E. heterolobum</i>	C775	Y.G. Wei Wei054 (IBK)	Guangxi, China	OR577343*	OR568593*	OR568587	OR591474*
<i>E. oblongifolium</i>	C199	Y.G. Wei & F. Wen 1147 (IBK)	Guizhou, China	OR577338*	OR568588*	OR568582*	OR591469*
	C067	Y.G. Wei & L.F. Fu 068 (IBK)	Guangxi, China	OR577336*	OR568589*	OR568581*	OR591478*
<i>E. radicans</i>	C694	F. Wen 0111 (IBK)	Guizhou, China	OR577342*	OR568599*	OR568586*	OR591472*
<i>E. retrohirtum</i>	C598	A.K. Monro &Y.G. Wei AM6801 (IBK)	Guangxi, China	OR577340*	OR568591*	OR568579*	OR591470*
	C610	L.F. Fu & S.L. Huang FL0026 (IBK)	Yunnan, China	OR577341*	OR568592*	OR568580*	OR591471*
<i>E. robustipes</i>	C041	Y.G. Wei & L.F. Fu 002 (IBK)	Guangxi, China	OR577339*	OR568598*	OR568578*	OR591477*
<i>E. scaposum</i>	C085	F. Wen WF0068 (IBK)	Guizhou, China	OR577337*	OR568590*	/	OR591479*

made based on the morphological diagnosis of three putative species distinguishing from their closest relatives (Wang 2010b; Yang et al. 2011; Wei et al. 2012).

Genomic DNA extraction, PCR amplification & sequencing

Four universal barcodes: the nuclear ribosomal internal transcribed spacer (ITS) region, the *trnH-psbA* intergenic spacer, *matK* and *rbcL* were used to establish hypotheses of phylogenetic relationship based on their ability to detect variation at the species level (China Plant BOL Group et al. 2011; Gao et al. 2012). The primers used to amplify four universal barcodes were those developed by the Kress et al. (2005), China Plant BOL Group et al. (2011) and Gao et al. (2012). Total genomic DNA was isolated from dried plant material using a modified CTAB protocol (Chen et al. 2014). PCR amplification protocols followed Gao et al. (2012) and Fu et al. (2019).

Sequence alignment and phylogenetic analysis

Sequence data were edited and assembled using Lasergene Navigator (DNASTar, Madison, Wisconsin, USA). Cleaned sequences were then aligned with the MEGA 5.1 (Tamura et al. 2011) with additional manual refinements where necessary. Phylogenetic analyses for the aligned matrix were performed by maximum parsimony (MP), Bayesian inference (BI) and maximum likelihood (ML) methods. MP analyses were carried out using PAUP* 4.0b10 (Swofford 2002). All characters were unordered and equally weighted, and gaps were coded as missing data. Heuristic searches were performed using a starting tree built from stepwise addition with tree bisection-reconnection (TBR) branch swapping and 1,000 random addition replicates. To assess confidence in clades, 1,000 bootstrap replicates (maximum parsimony bootstrap; MPBS) with 10 random additions per replicate were used. The ML analyses were constructed in IQtree v1.6.12 (Nguyen et al. 2015) with 1,000 bootstrap replicates (MLBS) and HKY+G selected as the best model. The BI analyses were done using MrBayes v3.2.7a (Ronquist et al. 2012). The model of best fit (TIM+F+G4) was determined based on Bayesian information criterion (BIC) (Aho et al. 2014) in jModelTest2 v. 2.1.7 (Darriba et al. 2012). Two independent runs were performed, each consisting of four Markov Chain Monte Carlo (MCMC) chains. The beginning 25% of trees were discarded as burn-in while the remaining trees were used for generating a consensus tree to estimate posterior probabilities (PP). The convergence of the MCMC chains of each run was determined when the average standard deviation of split frequencies (ASDSF) achieved ≤ 0.01 .

Estimates of support

We adopted the same criteria of ML, MP and BI analyses as Tseng et al (2019): For ML and MP analyses, 70–79%, 80–89%, and 90–100% bootstrap supports were considered as weakly, moderately, and strongly supported, respectively, and values lower than 70% were considered as providing no support. For BI analyses, the posterior probabilities of < 0.9 , 0.9–0.94, 0.95–0.99, and 1.0 were considered as providing no, weak, moderate, and strong support, respectively.

Results

Morphological comparison

After consulting protologues, checking type specimens and other specimens as well as observing field individuals, a suite of morphological characters was confirmed and used to compare three putative species and their closest relatives. Characters used were plant height, stem indumentum, leaf shape, leaf indumentum, leaf venation, stipule shape, male inflorescence insertion, male inflorescence peduncle length, male bract appendage, male bracteole shape and male flower sepal number. Specifically, *Elatostema robustipes* had densely hispid stem comprising weakly curved to crooked, appressed hairs, broader half auriculate leaf basal, 3.5–4.5 mm male inflorescence peduncle length, 5 longitudinal ribs outer bracts and 1 longitudinal rib inner bracts, and oblanceolate or obovate male bracteoles that can be easily distinguished from *E. balansae* Gagnep., but showed no significant difference to *E. retrohirtum* Dunn (Table 2). *Elatostema scaposum* presented 50–90 cm plant height, male inflorescence borne on modified or unmodified stems and 5–15 mm male inflorescence peduncle length showing no significant difference to *E. oblongifolium* Fu ex W.T.Wang (Table 3). *Elatostema conduplicatum* had glabrous leaf blade, semitriplinerve leaf venation, triangular stipule, broadly triangular and corniculate male bract, linear-cymbiform and corniculate male bracteoles and 5-merous male flower which can be easily distinguished from *E. shanglinense*, but showed no significant difference to *E. coriaceifolium* W.T.Wang (Table 4).

Molecular analysis

The combined matrix had a length of 2,526 characters (ITS: 652, *trnH-psbA*: 375, *matK*: 800, *rbcL*: 699). Including indels, 289 (11.4%) were variable and 205 (8.1%) were parsimoniously informative. Phylogenies reconstructed using ML, MP and BI methods recovered consistent topologies for all samples of the in-group taxa, which formed a monophyletic clade with strong supports (MLBS

Table 2. Morphological comparison of *Elatostema robustipes*, *E. balansae* and *E. retrohirtum*.

Characters	<i>E. robustipes</i>	<i>E. balansae</i>	<i>E. retrohirtum</i>
Stem indumentum	densely hispid, weakly curved to crooked	glabrous or pubescent	densely hispid, weakly curved to crooked
Leaf shape	broader-half auriculate	broader-half broadly cuneate or rounded	broader-half auriculate
Male inflorescence peduncle length	3.5–4.5 mm	1.0–2.0 mm	4.0–6.0 mm
Male bract appendage	outer bracts bearing 5 longitudinal ribs; inner bracts bearing 1 longitudinal rib	outer bracts bearing 3 inconspicuous longitudinal ribs; inner bracts not bearing rib	outer bracts bearing 5–6 longitudinal ribs; inner bracts bearing 1–3 longitudinal ribs
Male bracteoles shape	oblanceolate or obovate	linear	Oblanceolate

Table 3. Morphological comparison of *Elatostema scaposum* and *E. oblongifolium*.

Characters	<i>E. scaposum</i>	<i>E. oblongifolium</i>
Plant height	50–90 cm	20–90 cm
Disposition of male inflorescence	borne on modified or unmodified stems	borne on unmodified stems
Male inflorescence peduncle length	5–15 mm	0.5–10 mm

Table 4. Morphological comparison of *Elatostema conduplicatum*, *E. shanglinense* and *E. coriaceifolium*.

Characters	<i>E. conduplicatum</i>	<i>E. shanglinense</i>	<i>E. coriaceifolium</i>
Leaf indumentum	glabrous	shortly ciliated	Glabrous
Leaf venation	semitriplinerve	triplinerve	Semitriplinerve
Stipule shape	triangular	narrowly lanceolate	triangular or narrowly ovate
Male bract shape	broadly triangular, conduplicate	oblong-cymbiform, not conduplicate	broadly ovate, conduplicate
Male bracteoles shape	linear-cymbiform, corniculate	narrowly linear, not corniculate	linear-cymbiform, corniculate
Male flower merism	5	4	5

100, MPBS 100, PP 1.0) sister to the outgroup taxa. The ingroup taxa were recovered as three subclades (A–C), each of which comprised the putative species and its most morphologically similar congener in a paraphyletic grouping (Fig. 1). In detail, clade A comprised three species including *Elatostema balansae*, *E. retrohirtum* and *E. robustipes* with strong supports (MLBS 100, MPBS 100, PP 1.0). *Elatostema balansae* was sister to a clade consist of *E. retrohirtum* and *E. robustipes*, whereas *E. robustipes* was nested in this clade including a paraphyletic *E. retrohirtum*, with strong supports in BI analysis (PP = 1). Similar situations also occurred in clade B and clade C that *E. scaposum* and *E. conduplicatum* were nested in the paraphyletic *E. oblongifolium* and *E. coriaceifolium*, respectively with strong supports (MLBS 98/100, MPBS 100/100, PP 1.0/1.0).

Discussion

Elatostema robustipes was described based on a single collection with two duplicate specimens that displayed only male inflorescences, with reference to *E. balansae* (Wei et al. 2012). It was argued that *E. robustipes* could be distinguished from *E. balansae*, based on leaf shape, male inflorescence peduncle length, bract appendage and bracteole shape (Table 2). At the time of

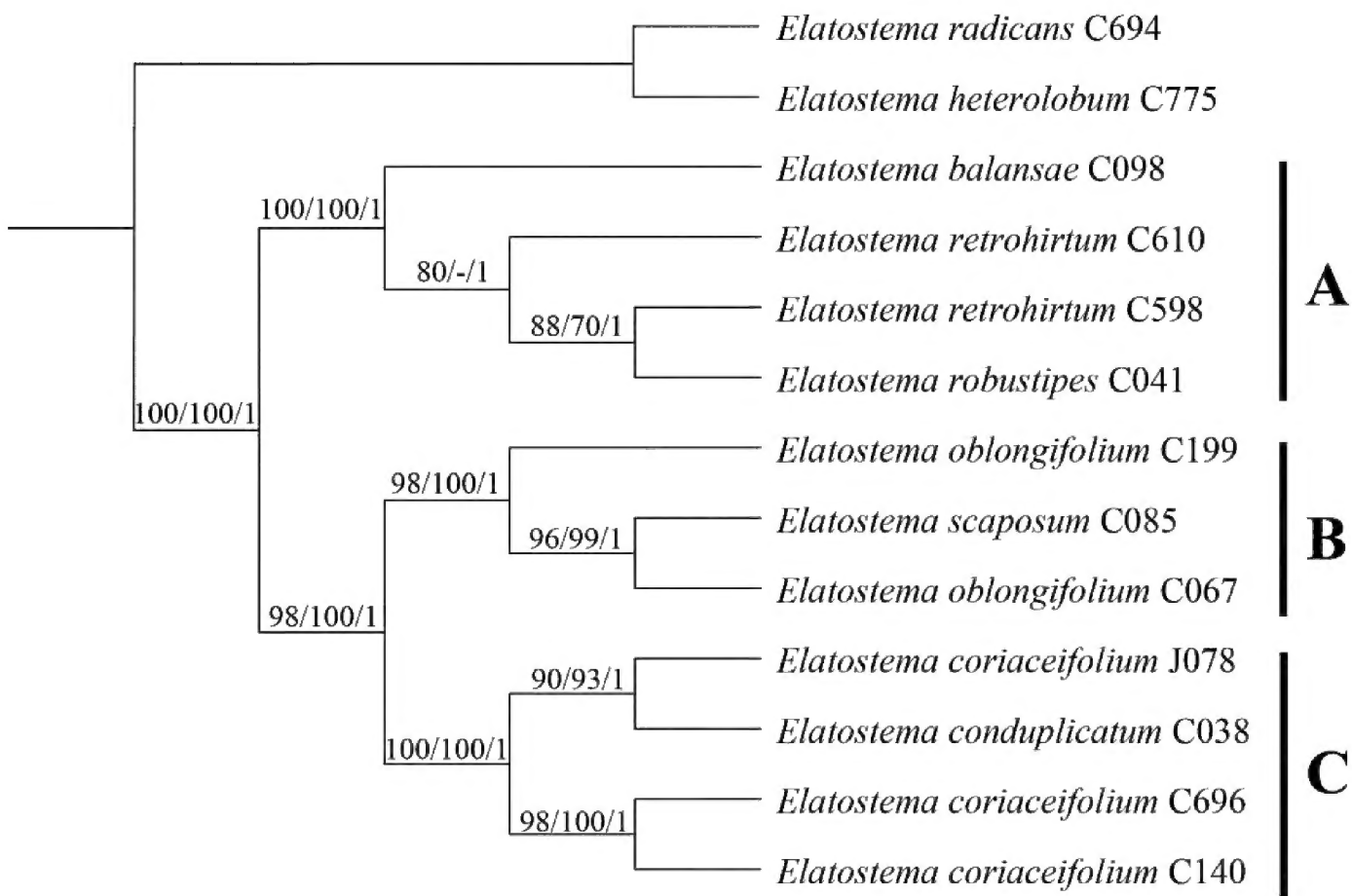


Figure 1. Phylogenetic tree of *Elatostema* generated by maximum likelihood (ML) of the combined dataset (ITS, *trnH-psbA*, *matK*, *rbcL*). Numbers on branches indicate bootstrap values ($\geq 60\%$) of ML and maximum parsimony (MP) analyses and posterior probability (≥ 0.8) of Bayesian inference (BI).

description, *E. robustipes* was not compared to another morphologically similar species, *E. retrohirtum*, as no description of the male inflorescence for the latter was known, limiting the basis of a comparison. During subsequent fieldtrips to Yunnan and northern Vietnam, we collected several specimens of *E. retrohirtum* bearing male inflorescences (Fu et al. 2014). These suggested that *E. robustipes* was, in fact, most similar to *E. retrohirtum*. A detailed morphological comparison of *E. robustipes* and *E. retrohirtum* showed that they share several diagnostic morphological characters: a densely hispid stem comprising weakly curved to crooked, appressed hairs; a male inflorescence with an involucre comprising six bracts, the outer two of which are larger and bear five longitudinal ribs, the four inner of which are smaller and bear a single longitudinal rib (Table 2; Fig. 2). Our phylogenetic analyses suggest that *E. robustipes* is nested in a clade A, which includes a paraphyletic *E. retrohirtum*, with strong supports in BI analysis (PP 1.0), and which itself is sister to a morphologically distinct, *E. balansae*. Based on the morphological and molecular evidence, we consider that *E. robustipes* and *E. retrohirtum* represent the same species. The latter name has priority.

Elatostema scaposum was described with reference to *E. oblongifolium*, based on 12 duplicate specimens (Yang et al. 2011). The diagnostic characters in the diagnosis were plant height, male inflorescence peduncle length and the disposition of the male inflorescences (Yang et al. 2011). Based on our extensive field observations and collections across Guizhou and Yunnan, plant height and male inflorescence peduncle length for both groupings overlapped, and a single population of *E. scaposum* being recovered which comprises individuals bearing male inflorescences on both modified and unmodified stems (Table 3; Fig. 2) suggesting that the character of modified stem is not stable for the species. Our phylogenetic analyses recovered *E. scaposum* nested in clade B, which includes a paraphyletic *E. oblongifolium*, with strong support (MLBS 98, MPBS 100, PP 1.0). Based on the morphological and molecular evidence, we consider that *E. scaposum* and *E. oblongifolium* represent the same species. The latter name has priority.

Elatostema conduplicatum was described from two duplicate specimens of a single collection comprising only male inflorescences, with reference to *E. shanglinense* (Wang 2010b). *Elatostema conduplicatum* can easily be distinguished from *E. shanglinense* by a suite of characters, including leaf indumentum and venation, stipule shape, male bract shape and conduplicate arrangement, male bracteole shape, and male flower merism (Table 4). *Elatostema conduplicatum* was not, however, compared with another species, *E. coriaceifolium*, presumably as the male inflorescences of the latter were unknown at the time. Wang (2010c) later provided a supplementary description of the male inflorescences of *E. coriaceifolium*, which suggested that *E. conduplicatum* was most similar to *E. coriaceifolium*, from which it could be distinguished by the conduplicate bract arrangement. Our field observations, however, clearly showed that the male bract of *E. coriaceifolium* is also conduplicate (Table 4, Fig. 2). In addition, our phylogenetic analyses suggest that *E. conduplicatum* is nested within clade C, which includes a paraphyletic *E. coriaceifolium*, with strong supports (MLBS 100, MPBS 100, PP 1.0). Based on the morphological and molecular evidence, we consider that *E. conduplicatum* and *E. coriaceifolium* represent the same species. The latter name has priority.

Based on the above, we present the following detailed taxonomic treatments for the species.

Taxonomic treatment

***Elatostema coriaceifolium* W.T.Wang, Acta Phytotax. Sin. 31(2): 170. 1993.**

= *Elatostema conduplicatum* W.T.Wang, Guihaia 30(1): 3. 2010. Syn. nov. Type: CHINA. Guangxi: Donglan County, Bala, Y.M. Shui & W.H. Chen B2004-171A (holotype: PE [PE01842427!]; isotype: KUN!).

Type. CHINA. Guizhou: Libo, Wengangmogan, 29 April 1984, Q.H. Chen et al. 2289 (holotype: HGAS).

Description. *Perennial herb*. Stems 140–185(–270) × 1.0–1.5 mm, erect, simple, fasciculate, furfuraceous, glabrous; stipules 2, triangular or narrowly ovate, 0.8–1.8 × 0.4–1.0 mm, without cystoliths. Leaves sessile or shortly petiolate, glabrous; laminae 12–24(–40) × 8–11(–14) mm, obliquely elliptic or rhombic-elliptic, chartaceous or thinly coriaceous, semitriplinerve; cystoliths densely scattered; base asymmetrical, broader-half auriculate, narrower-half cuneate; margin denticulate; apex acute. Staminate inflorescences solitary, capitate; sessile; receptacle inconspicuous, subtended by marginal bracts; the bracts 6, unequal, outer 2 bracts major, broadly ovate, conduplicate, 4–4.5 × ca. 3 mm, abaxial surface with 1 longitudinal rib, ribbed extending apically as a corniculate protuberance, inner 4 bracts minor, oval, ca. 3 × 2–2.8 mm, abaxial surface with 1 longitudinal rib, ribbed extending apically as a corniculate protuberance, glabrous; staminate flowers pedicellate; bracteoles 2 per flower, subequal, ca. 2.4 × 1.0 mm, linear-cymbiform, subapical appendage, corniculate, shortly ciliate; tepals 5, broadly ovate, subapical appendage 0.8–1.1 mm, corniculate, pubescent. Pistillate inflorescence solitary, capitate; peduncle ca. 1 mm, glabrous; receptacle broadly rectangular, 3–3.5 mm, glabrous, subtended by marginal bracts, the bracts ca. 20, unequal, outer 6 bracts major, triangular or broadly ovate, ca. 1.1 × 0.4–2 mm, subapical appendage, inner bracts minor, narrowly triangular or linear, sparsely ciliate or glabrous; pistillate flowers pedicellate; bracteoles 2 per flower, equal, 0.9–1.5 × 0.1–0.4 mm, linear, ciliate or glabrous; achenes ca. 0.8 mm, narrowly ellipsoidal, ca. 6–8-ribbed.

Distribution. This species is endemic to China (Guangxi, Guizhou).

***Elatostema oblongifolium* Fu ex W.T.Wang, Bull. Bot. Lab. N. E. Forest. Inst., Harbin 7: 26. 1980.**

≡ *Pellionia bodinieri* H. Lév., Repert. Spec. Nov. Regni Veg. 11: 551. 1913.

≡ *Elatostema bodinieri* (H.Lév.) Hand.-Mazz., Symb. Sin. Pt. 7: 144. 1929, nom. illeg., non H.Lév. 1913. Type: CHINA. Guizhou: Gan-pin, 29 April 1897, Bodinier 1547 (syntype E); Ou-la-gay, 9 April 1898, Seguin s.n. (syntype: E).

= *Elatostema schizocephalum* W.T.Wang, Bull. Bot. Lab. N. E. Forest. Inst., Harbin 7: 82. 1980. Type: CHINA. Hunan: Yizhang, 22 January 1942, S.Q. Chen 73 (holotype: PE [PE00023194!]). = *Elatostema multicanaliculatum* B.L.Shih & Yuen P. Yang, Bot. Bull. Acad. Sin. 36: 268. 1995. Type: CHINA. Taiwan: Taoyuan Co., Mt. Lala, 23 October 1994, B.L. Shih 3226 (isotypes: HAST, TAI!, TAIF).

= *Elatostema scaposum* Q. Lin & L.D. Duan, Nordic J. Bot. 29: 420. 2011. Syn. nov. Type: CHINA. Guizhou: Libo County, Jialiang Baibidong, alt. 800 m, 26

October 2003, Q. Lin & L.D. Duan 1023 (holotype: PE [PE01863021!]; isotypes E!, GH, HUH [HUH A00293663!], K, L, NY, PE [PE01863023!, PE01863023!], TUS, US, WU).

Description. *Perennial herb*. Stems 20–90 × 0.5–12 mm, ascending or erect, branched or simple, with 5 or more longitudinal canals, glabrous; stipules 2, narrowly triangular to subulate or narrowly lanceolate, 2.5–12 × 0.2–2.0 mm,

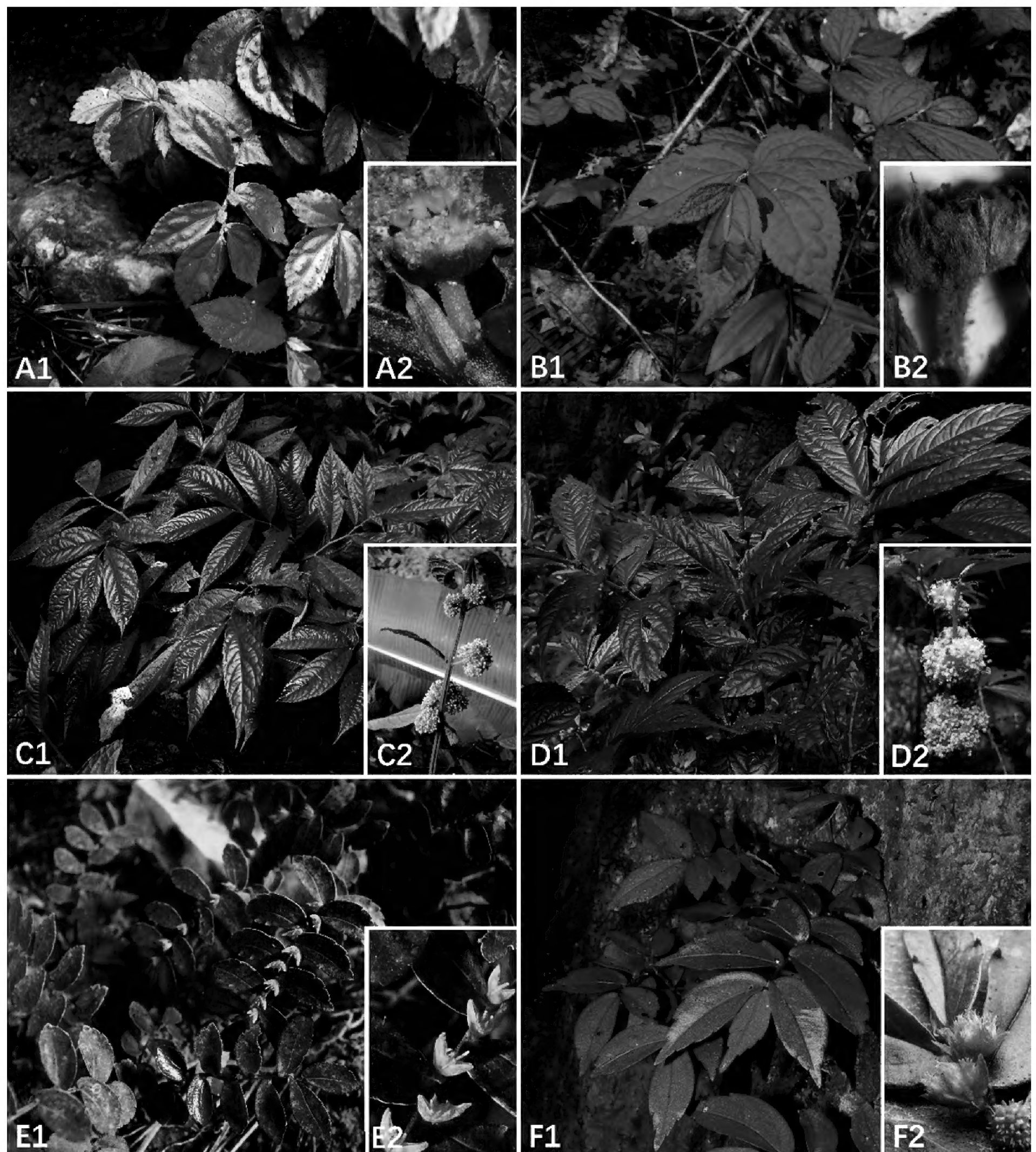


Figure 2. *Elatostema* spp. **A** *E. retrohirtum* **B** *E. robustipes* **C** *E. oblongifolium* **D** *E. scaposum* **E** *E. coriaceifolium* **F** *E. conduplicatum* **1** habit **2** male inflorescence.

glabrous. Leaves sessile or short petiolate; laminae 50–220 × 14–50(–80) mm, obliquely oblong or elliptic, chartaceous, pinnately nerved; cystoliths densely scattered; base asymmetrical, broader-half rounded to cordate, narrower-half cuneate; margin serrulate to coarsely serrate; apex acuminate or long acuminate. Staminate inflorescences borne on modified or unmodified stems, solitary, cymiferous, shortly pedunculate, subglabrous; bracts membranous, ovate, lanceolate or linear, 2–12 mm, glabrous; staminate flowers pedicellate, glabrous; tepals 5, narrowly elliptic, ca. 2 mm, subapical appendage, shortly corniculate. Pistillate inflorescence paired, capitate; peduncle ca. 3 mm, glabrous; receptacle rectangle or broadly ovate, deeply divided into two lobes, lobe further weakly divided into two lobes, 2–10 × 3 mm, glabrous, subtended by marginal bracts, the bracts 25 or more, unequal, outer bracts major, triangular, 0.6–1.0 × 0.4–0.8 mm, glabrous, inner bracts minor, linear or lanceolate, sparsely ciliate; pistillate flowers pedicellate; bracteoles 2 per flower, subequal, 0.5–1.5 mm, linear, narrowly obovate or cymbiform; achenes 0.6–0.9 × 0.3–0.5 mm, broadly ellipsoidal or ovoid, ca. 6-ribbed.

Distribution. This species is distributed in China (Chongqing, Hubei, Hunan, Guangxi, Guizhou, Sichuan, Taiwan, Yunnan) and Vietnam (Ha Giang).

***Elatostema retrohirtum* Dunn, Bull. Misc. Inform. Kew, Addit. Ser. 10: 249. 1912.**

= *Elatostema robustipes* W.T.Wang, F.Wen & Y.G.Wei, Ann. Bot. Fenn. 49: 188. 2012. Syn. nov. Type: CHINA. Guangxi: Huanjiang County, Mulun National Reserve, Hongdong, alt. 308–512 m, 24°43'N, 108°18'E, 26 April 2009, Y.G. Wei 124 (holotype: IBK!; isotypes: IBK!, PE [PE01843378!, PE01843379!]).

Type. CHINA. Guangdong: near Yit-hai Han valley, *Dunn's Han Exped., Herb. Hongk. no. 6288* (holotype: K!).

Description. Perennial herb. Stems 150–350 × 1.8–2.5 mm, ascending or erect, branched, densely hispid, the hairs weakly curved to crooked, appressed; stipules 2, linear-lanceolate, 4–8 × 1.0–2.0 mm, cystoliths sparsely scattered, glabrous. Leaves sessile or short petiolate, petioles 0–1(–4.5) mm, densely hispid, the hairs weakly curved to crooked, appressed; laminae 40–60(–100) × 15–20(–50) mm, obliquely elliptic, herbaceous or chartaceous, triplinerve; cystoliths densely scattered; base asymmetrical, broader-half rounded or auriculate, narrower-half cuneate; margin denticulate; apex short acuminate or acute, rarely acuminate. Staminate inflorescences solitary, capitate; peduncle 3.5–6.0 × 0.3–0.8 mm, sparsely hispid, the hairs weakly curved, appressed; receptacle 2–4 × 3–5 mm, rectangle or oblong, glabrous, subtended by marginal bracts; the bracts ca. 6, unequal, outer 2 bracts major, broadly ovate, 2–2.5 × 4–5 mm, abaxial surface sparsely hispid, the hairs weakly curved, appressed, with 5 or 6 longitudinal ribs, each ribbed extending apically as a corniculate protuberance, inner 4 bracts minor, obovate, ca. 2 × 3 mm, abaxial surface with 1–3 longitudinal ribs, at least one ribbed extending apically as a corniculate protuberance, glabrous; staminate flowers pedicellate, glabrous; bracteoles 2 per flower, equal, 2.5–4.0 × 1.0–1.5 mm, oblanceolate or obovate, glabrous; tepals 4, ovate, 0.9–1.2 × 0.7–0.9 mm, subapical appendage ca. 0.5 mm, corniculate, glabrous. Pistillate inflorescence solitary, capitate; peduncle ca. 1 × 0.5 mm, glabrous;

receptacle subrounded, 3–3.5 mm in diam., glabrous, subtended by marginal bracts, the bracts numerous, subequal, triangular, 0.6–1.2 × 0.4–0.6 mm; pistillate flowers pedicellate; bracteoles 2 per flower, subequal, ca. 0.6–1.2 × 0.3 mm, spatulate-linear; achenes 0.5–0.6 mm, ovoid or ellipsoidal, ca. 6-ribbed.

Distribution. This species is distributed in China (Guangdong, Guangxi, Guizhou, Sichuan, Yunnan) and Vietnam (Bac Kan, Gia Lai, Ha Giang, Ha Noi, Hai Phong, Hoa Binh, Lam Dong, Nghe An, Ninh Binh, Son La, Tuyen Quang).

Conclusions

We conclude that the three species studied are conspecific to earlier described taxa that have priority under the International Code of Nomenclature for Algae, Fungi and Plants and place them in synonymy with these names. Our results emphasize that some morphological characters, such as the number, shape and size of bract and bracteole, are relatively constant (Wang 2010a), whilst the bearing of male inflorescences on modified stems is unstable and ill-suited to delimit species in *Elatostema*. More importantly, our results provide further support for the need to integrate multiple lines of evidence when describing new species based on very small numbers of individuals, as is frequently the case for point-endemic species (Hong 2016; Fu et al. 2021).

Acknowledgements

We are grateful to the curators at BM, E, IBK, IBSC, PE and VNMN for their help on checking specimens. We are also grateful to Prof. Fang Wen, Chi Xiong and Xin-Yang He for participating in fieldwork.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This study was supported by Guangxi Natural Science Foundation Program (2022GXNS-FAA035440), the National Natural Science Foundation of China (32160082, 32360297), the Fund of Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain (22-035-26), the Science Research Foundation of Guangxi Institute of Botany (Guizhiye 23004) and the Basic Research Fund of Guangxi Academy of Sciences (CQZ-C-1901).

Author contributions

Data curation: RFW. Funding acquisition: LFF. Investigation: AKM, ZBX, RFW. Project administration: LFF. Writing – original draft: ZBX, LFF. Writing – review and editing: AKM, LFF.

Author ORCIDs

Zi-Bing Xin  <https://orcid.org/0000-0002-0062-6930>

Long-Fei Fu  <https://orcid.org/0000-0001-8708-4718>

Data availability

All of the data that support the findings of this study are available in the main text.

References

- Aho K, Derryberry D, Peterson T (2014) Model selection for ecologists: The worldviews of AIC and BIC. *Ecology* 95(3): 631–636. <https://doi.org/10.1890/13-1452.1>
- Chen LY, Song MS, Zhu HG, Li ZM (2014) A modified protocol for plant genome DNA extraction. *Plant Diversity and Resources* 36: 375–380.
- China Plant BOL Group, Li DZ, Gao LM, Li HT, Wang H, Ge XJ, Liu JQ, Chen ZD, Zhou SL, Chen SL, Yang JB, Fu CX, Zeng CX, Yan HF, Zhu YJ, Sun YS, Chen SY, Zhao L, Wang K, Yang T, Duan GW (2011) Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proceedings of the National Academy of Sciences of the United States of America* 108(49): 19641–19646. <https://doi.org/10.1073/pnas.1104551108>
- Clements R, Sodhi NS, Schithuizen M (2006) Limestone karsts of Southeast Asia: Imperiled arks of biodiversity. *Bioscience* 56(9): 733–742. [https://doi.org/10.1641/0006-3568\(2006\)56\[733:LKOSAI\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2006)56[733:LKOSAI]2.0.CO;2)
- Darriba D, Taboada GL, Doallo R, Posada D (2012) JModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* 9(8): e772. <https://doi.org/10.1038/nmeth.2109>
- Duan LD, Lin Q (2003) Taxonomic notes on *Elatostema xinningense* W.T.Wang. *Acta Phytotaxonomica Sinica* 41(5): 495–496.
- Duan LD, Lin Q, Shao Q (2006) Two new synonyms of *Elatostema* (Urticaceae) in Hunan, China. *Zhiwu Fenlei Xuebao* 44(4): 474–476. <https://doi.org/10.1360/aps050171>
- Fu LF, Huang SL, Liu Y, Do VT, Wen F, Wei YG (2014) A newly recorded species of *Elatostema* (Urticaceae) from Vietnam. *Taiwania* 59(3): 281–286. <https://doi.org/10.6165/tai.2014.59.281>
- Fu LF, Su LY, Mallik A, Wen F, Wei YG (2017) Cytology and sexuality of 11 species of *Elatostema* (Urticaceae) in limestone karsts suggests that apomixis is a recurring phenomenon. *Nordic Journal of Botany* 35(2): 251–256. <https://doi.org/10.1111/njb.01281>
- Fu LF, Monro AK, Wen F, Xin ZB, Wei YG, Zhang ZX (2019) The rediscovery and delimitation of *Elatostema setulosum* W.T.Wang (Urticaceae). *PhytoKeys* 126: 79–88. <https://doi.org/10.3897/phytokeys.126.35707>
- Fu LF, Monro AK, Yang TG, Wen F, Pan B, Xin ZB, Zhang ZX, Wei YG (2021) *Elatostema qinzhouense* (Urticaceae), a new species from limestone karst in Guangxi, China. *PeerJ* 9: e11148. <https://doi.org/10.7717/peerj.11148>
- Gao LM, Liu J, Cai J, Yang JB, Zhang T, Li DZ (2012) A synopsis of technical notes on the standards for plant DNA barcoding. *Plant Diversity and Resources* 34(6): 592–606. <https://doi.org/10.3724/SP.J.1143.2012.12138>
- Hao Z, Kuang YW, Kang M (2015) Untangling the influence of phylogeny, soil and climate on leaf element concentrations in a biodiversity hotspot. *Functional Ecology* 29(2): 165–176. <https://doi.org/10.1111/1365-2435.12344>
- Hollingsworth PM, Forrest LL, Spouge JL, Hajibabaei M, Ratnasingham S, van der Bank M, Chase MW, Cowan RS, Erickson DL, Fazekas AJ, Graham SW, James KE, Kim K-J, Kress WJ, Schneider H, van AlphenStahl J, Barrett SCH, van den Berg C, Bogarin D, Burgess KS, Cameron KM, Carine M, Chacón J, Clark A, Clarkson JJ, Conrad F, Devey DS, Ford CS, Hedderson TAJ, Hollingsworth ML, Husband BC, Kelly LJ, Kesanakurti PR, Kim JS, Kim Y-D, Lahaye R, Lee H-L, Long DG, Madriñán S, Maurin O, Meusnier I, Newmaster

- SG, Park C-W, Percy DM, Petersen G, Richardson JE, Salazar GA, Savolainen V, Seberg O, Wilkinson MJ, Yi D-K, Little DP, CBOL Plant Working Group (2009) A DNA barcode for land plants. *Proceedings of the National Academy of Sciences of the United States of America* 106(31): 12794–12797. <https://doi.org/10.1073/pnas.0905845106>
- Hollingsworth PM, Graham SW, Little DP (2011) Choosing and using a plant DNA barcode. *PLoS ONE* 6(5): e19254. <https://doi.org/10.1371/journal.pone.0019254>
- Hong D (2016) Opinion of raising rationality in species delimitation. *Biodiversity Science* 24(3): 360–361. <https://doi.org/10.17520/biods.2016075>
- Jiang ZC, Lian YQ, Qin XQ (2014) Rocky desertification in Southwest China: Impacts, causes, and restoration. *Earth-Science Reviews* 132: 1–12. <https://doi.org/10.1016/j.earscirev.2014.01.005>
- Kress JW, Wurdack KJ, Zimmer EA, Weigt LA, Janzen DH (2005) Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences of the United States of America* 102(23): 8369–8374. <https://doi.org/10.1073/pnas.0503123102>
- Lin Q, Duan LD (2003) Taxonomic notes on five species of *Elatostema* (Urticaceae) from China. *Acta Botanica Yunnanica* 25(6): 633–638.
- Lin Q, Friis I, Wilmot-Dear MC (2003) *Elatostema*. In: Wu Z, Raven PH (Eds) *Flora of China* (Vol. 5). Science Press, Beijing and Missouri Botanical Garden Press, St. Louis, Missouri, 76–189.
- Lin Q, Duan LD, Yang ZR, Shui YM (2011) Notes on *Elatostema* section *Androsyce* Wedd. (Urticaceae). *Journal of Systematics and Evolution* 49(2): 163–163. https://doi.org/10.1111/j.1759-6831.2011.00121_4.x
- Nguyen LT, Schmidt HA, Von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32(1): 268–274. <https://doi.org/10.1093/molbev/msu300>
- Parise M, De Waele J, Gutierrez F (2009) Current perspectives on the environmental impacts and hazards in karst. *Environmental Geology* 58(2): 235–237. <https://doi.org/10.1007/s00254-008-1608-2>
- POWO (2023) Plants of the World Online. Royal Botanic Gardens, Kew. <http://www.plantsoftheworldonline.org/> [Accessed 16 October 2023]
- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61(3): 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Swofford DL (2002) PAUP*: phylogenetic analysis using parsimony (* and other methods), v. 4.0 beta 10. Sinauer Associates, Sunderland.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28(10): 2731–2739. <https://doi.org/10.1093/molbev/msr121>
- Tseng YH, Monroe AK, Wei YG, Hu JM (2019) Molecular phylogeny and morphology of *Elatostema* s.l. (Urticaceae): Implications for inter- and infra-generic classification. *Molecular Phylogenetics and Evolution* 132: 251–264. <https://doi.org/10.1016/j.ympev.2018.11.016>
- Wang WT (2010a) Morphology of the bracts of *Elatostema* (Urticaceae) and the evolutionary trends in them. *Guihaia* 30(5): 571–583.
- Wang WT (2010b) New taxa of *Pellionia* and *Elatostema* (Urticaceae) from China. *Guihaia* 30(1): 1–12.
- Wang WT (2010c) Notes on the genus *Elatostema* (Urticaceae). *Guihaia* 30(6): 713–727.

- Wang WT (2014) *Elatostema* (Urticaceae) in China. Qingdao Press, Qingdao.
- Wei YG (2018) The Distribution and Conservation Status of Native Plants in Guangxi, China. China Forestry Publishing House, Beijing, 876 pp.
- Wei YG, Monro AK, Wang WT (2011) Additions to the Flora of China: Seven new species of *Elatostema* (Urticaceae) from the karst landscapes of Guangxi and Yunnan. *Phytotaxa* 29(1): 1–27. <https://doi.org/10.11646/phytotaxa.29.1.1>
- Wei YG, Wen F, Wang WT (2012) *Elatostema robustipes* (Urticaceae), a new species from Guangxi, and *Pellionia tenuicuspis* (Urticaceae), a new species from Guangdong, China. *Annales Botanici Fennici* 49(3): 188–192. <https://doi.org/10.5735/085.049.0305>
- Wei YG, Do VT, Wen F (2022) A Checklist to the Plants of Northern Vietnam. China Forestry Publishing House, Beijing, 606 pp.
- Wells T, Maurin O, Dodsworth S, Friis I, Cowan R, Eritawale N, Brewer G, Forest F, Baker WJ, Monro AK (2021) Combination of Sanger and target-enrichment markers supports revised generic delimitation in the problematic ‘*Urera* clade’ of the nettle family (Urticaceae). *Molecular Phylogenetics and Evolution* 158: e107008. <https://doi.org/10.1016/j.ympev.2020.107008>
- Xu ZR (1995) A study of the vegetation and floristic affinity of the limestone forests in southern and southwestern China. *Annals of the Missouri Botanical Garden* 82(4): 570–580. <https://doi.org/10.2307/2399837>
- Yang ZR, Duan LD, Lin Q (2011) *Elatostema scaposum* sp. nov. (Urticaceae) from Guizhou, China. *Nordic Journal of Botany* 27(4): 420–423. <https://doi.org/10.1111/j.1756-1051.2010.00662.x>

Appendix 1

Specimens used for morphological studies.

- Elatostema conduplicatum*** W.T.Wang, **CHINA. Guangxi:** Donglan County, Bala, Y.M. Shui & W.H. Chen B2004-171A (KUN, PE [PE01842427]).
- Elatostema coriaceifolium*** W.T.Wang, **CHINA. Guangxi:** Fengshan County, Yangzi cave, 28 January 2018, Z.B. Xin XZB20180128-01 (IBK); **Guizhou:** Libo County, Jiarong Town, 21 October 2012, F. Wen 0097 (IBK); Dushan County, Jichang Town, 19 October 2012, F. Wen 0070 (IBK).
- Elatostema oblongifolium*** Fu ex W.T.Wang, **CHINA. Guizhou:** Anlong County, 29 October 2010, F. Wen 1082 (IBK); Dushan County, Jichang Town, 19 October 2012, F. Wen 0068 (IBK); **Yunnan:** Guangnan County, Sanla waterfall, 08 November 2011, F. Wen WFLTC111108 (IBK); Malipo County, Xiajinchang village, 23 October 2023, C. Xiong & X.Y. He XC20231023-06 (IBK).
- Elatostema retrohirtum*** Dunn, **CHINA. Guangdong:** near Yit-hai Han valley, Dunn’s Han Exped., Herb. Hongk. no. 6288 (K); **Guangxi:** Longzhou County, 20 May 2010, A.K. Monro & Y.G. Wei 6801 (IBK); **Yunnan:** between Malipo County and Babu Village, alt. 931 m, 23°14'52"N, 104°46'37"E, 2 May 2013, L.F. Fu & S.L. Huang FL0015 (IBK).
- Elatostema robustipes*** W.T.Wang, F.Wen & Y.G.Wei, **CHINA. Guangxi:** Huanjiang County, Mulun National Reserve, Hongdong, alt. 308–512 m, 24°43'N, 108°18'E, 26 April 2009, Y.G. Wei 124 (IBK, PE[PE01843378, PE01843379]).
- Elatostema scaposum*** Q.Lin & L.D.Duan, **CHINA. Guizhou:** Libo County, Jialiang Baibidong, alt. 800 m, 26 October 2003, Q. Lin & L.D. Duan 1023 (E, HUH [HUH A00293663], PE [PE01863021, PE01863023]).